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THE ABNORMAL CANTHARIDIN BLISTER IN ATOPY: AN EXPLANATION OF ITS PRODUCTION.

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It was shown by Allison and Bettley (1958) that a standard application of cantharidin to the normal skin of eczematous subjects caused blisters containing more protein than their own plasma and more than that of blisters similarly produced on the skin of normal subjects. These differences were found to be particularly marked in cases of atopic eczema and seemed to be due to some factor in the epidermis. It is possible that atopic eczema, like many other inherited diseases, will eventually prove to be due to an inborn deficiency, partial or complete, of one or more enzymes.

Inherited enzyme deficiencies may become manifest in several ways. Perhaps the commonest is the accumulation and excretion of metabolic intermediates in abnormal amounts, associated with an abnormal and usually typical clinical picture, as in phenylketonuria (Jervis, 1947, 1950 and 1953). Alternatively, the enzyme deficiency may present as an absence of a normal metabolic end product, as in albinism (Lerner and Fitzpatrick, 1950). Thirdly, an inborn enzyme deficiency state may be recognized by the association of a clinical syndrome with the absence of a particular enzyme on direct measurement, as in hypophosphatasia (Rathbun, 1948).

Atopic eczema, psoriasis, ichthyosis, Darier's disease and benign familial pemphigus are a few of the important inherited skin conditions which are

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possibly due to such inherited enzyme deficiencies, the exact nature of which has not so far been demonstrated.

A possible lead would be gained to determining such a relative enzyme deficiency in the case of atopy, if it could be shown that cantharidin inhibited a single enzyme system. It therefore seemed desirable to demonstrate the mechanism by which cantharidin produced epidermal lysis.

It has been known for many years that local applications of cantharidin to the skin result in an intraepidermal vesicle (Miescher, 1936). This epidermal change can be shown to be associated with prickle cell damage and subsequent acantholysis. Initially, a stage of intercellular oedema may be seen, similar to that found in a stage of eczema. Blister formation following friction is also intraepidermal, but is due to primary cell damage and is not associated with acantholysis or spongiosis (Naylor, 1955). Subepidermal blisters are produced by some well known vesicants, namely lewisite (Cameron, *et al.*, 1946), mustard gas (Koontz, 1944; McAdams, 1956) and by second degree burns (Moritz, 1947).

The toxic effect of mustard gas has been shown to be due to its inhibitory action on the enzyme hexokinase (Dixon and Needham, 1946). The principal inhibitory effect of lewisite is on the pyruvate oxidase system (Peters *et al.*, 1945) though other enzymes containing SH groups may be affected to a lesser extent. The arsenic of the lewisite molecule combines reversibly with dithiol groups of the enzyme protein molecule, thereby inactivating it.

The latter explanation of the action of cantharidin seemed unlikely since the proteolytic action of papain, activated by cyanide, on haemoglobin was not inhibited by cantharidin (Mills, 1958), i.e. cantharidin did not produce a stable inactive ring with papain. Similarly, the protease from *Proteus vulgaris* (which does not require activation by cyanide) was not affected by cantharidin. Besides, dimercaprol does not inhibit the vesicant action of cantharidin. This experiment was performed seven times, incorporating the dimercaprol in the lead plaster mass (B.P.C., 1934) base of the 0.2% cantharidin application and seven times by repeatedly applying dimercaprol for twenty minutes to the test site before applying the patch test of cantharidin. The skin was examined every two hours after the first application of the cantharidin plasters and no delay was noticed in the appearance of the blister as compared with a control site on the same limb of the same subject tested with 0.2% cantharidin alone. Applications 5 mm. in diameter were used in these tests.

The only recent study of the effect of cantharidin on whole tissue has been that of Haas *et al.* (1950), using mouse liver. They found that a 5×10^{-4} M solution of cantharidin produced 30% inhibition of respiration in 30 minutes and a 5×10^{-5} M solution produced 10% inhibition in 30 minutes. They also studied its affect on anaerobic glycolysis and found that a 5×10^{-4} M solution produced 30% inhibition in 60 minutes, whereas 1.6×10^{-4} M

resulted in 190% activation, and a 5×10^{-5} M solution caused 60% activation of anaerobic glycolysis in 60 minutes. The amount of liver used was 1–2 mg. dry weight, giving a wet weight of not more than 10 mg.; these are small amounts of tissue for the accurate determination of respiration by the conventional Warburg method.

Borger and Groll (1926) examined the effect of an alcoholic tincture of cantharidin on the respiration of mouse ears and found an increase in the rate of respiration. These results were partially invalidated by the use of an alcoholic extract of Spanish fly, which might have contained substances other than cantharidin.

MATERIALS AND METHODS.

Cantharidin and Di-sodium cantharidin.

Cantharidin is only sparingly soluble in water, 3.3 mg./100 ml. (0.0001 M) at room temperature (*Handbook of Chemistry and Physics*, 1950–1951). For enzyme studies, it is often necessary to have higher concentrations than could be obtained in water.

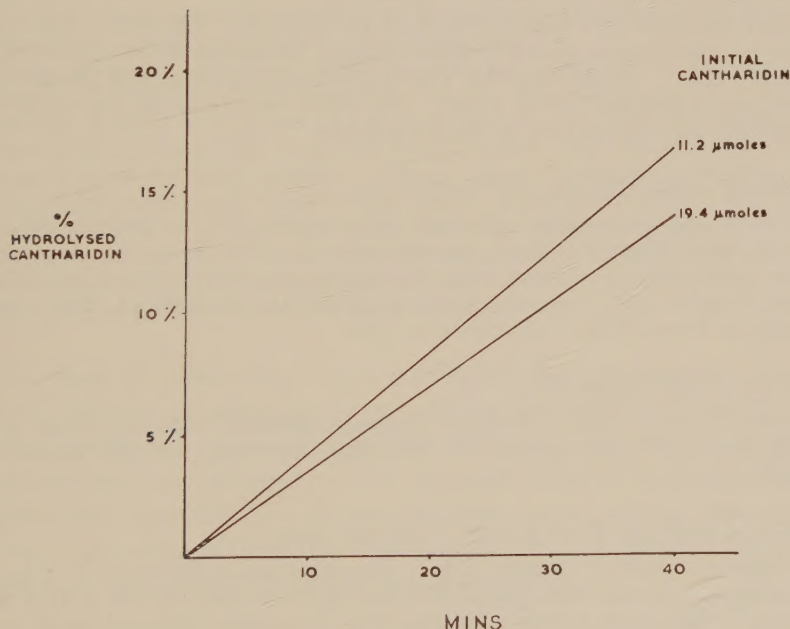
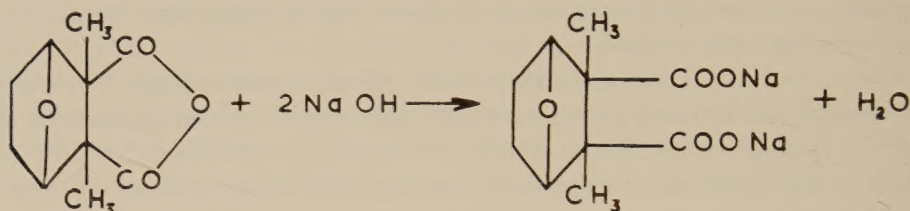


FIG. 1.

It was noted that in bicarbonate buffer, cantharidin was slowly hydrolyzed to the dibasic acid. This rate of hydrolysis was measured, and from the graph (Fig. 1), the hydrolysis constant was calculated (cf. Leloir, 1951). The average value for this constant was found to be $k = 1.75 \times 10^{-3}$. The finding that cantharidin underwent hydrolysis led to the preparation of a water-soluble di-sodium salt of cantharidin (DSC). This was shown to have the same vesicant properties in high dilution.

The structure and formula for cantharidin are: hexahydro-3a 7a-dimethyl-4-7-epoxy-isobenzofuran-1 : 3 dione, $C_{10}H_{12}O_4$, having a molecular weight of 196. The di-sodium salt is formed by the reaction



A weighed amount, 40 mg., of cantharidin (B.D.H.) was suspended in 5 ml. of water. This was titrated with 0.1 N NaOH at 37° C. using phenolphthalein as indicator. The NaOH was added in small amounts and the solution was continuously shaken until a permanent pink colour was observed. The amount of alkali required agreed with that expected from the neutralization of the two newly formed carboxyl groups.

Preparation of Rat Liver and Brain Cortex Slices.

The rats were killed by a blow on the back of the head. The tissues were removed as quickly as possible and placed in Krebs-Ringer phosphate saline (Umbreit, Burris and Stauffer, 1957). Slices were cut with a Stadie-Riggs slicer and transferred to a Petri dish containing the above saline. The slices were blotted and weighed on a torsion balance and transferred to Warburg flasks.

Preparation of Ascites Tumour Cell Suspensions.

The Ehrlich ascites tumour cells had been maintained in heterozygous albino mice, which were killed 7-9 days after inoculation with the ascites tumour. The cells were collected and washed with Krebs-Ringer bicarbonate saline (Umbreit, Burris and Stauffer, 1957) by centrifuging for 5 minutes at 2000 g. They were then resuspended in Krebs-Ringer bicarbonate saline.

Preparation of Yeast Suspensions.

Compressed baker's yeast (Distillers Company Limited) was suspended in media containing Na^+ , K^+ and glucose in strengths depending on the nature of the experiment.

Preparation of Cell Free Yeast Extract, "Lebedev Juice".

Fresh brewer's yeast ("Bottom Ale") was dried on a Buchner filter to remove excess moisture. It was then spread on filter paper to dry out for 2 days at room temperature. The yeast was then broken up in an electric mill and dried overnight *in vacuo* over P_2O_5 : 30 g. of this material was mixed with 3 vols. (90 ml.) of distilled water and incubated with occasional stirring at 37° C. in air. At the end of 2 hours, the suspension was centrifuged and the supernatant fluid was used as Lebedev juice.

Enzymes and Co-enzymes.

Phosphohexokinase was prepared from rat brain acetone powder according to the method of Muntz and Hurwitz (1951).

A dialyzed extract of rabbit muscle was used as the source of the triose phosphate dehydrogenase system (Roitt, 1956).

The following enzymes were obtained from C. F. Boehringer und Soehne, Mannheim, West Germany: lactic dehydrogenase, glutamic acid dehydrogenase, aldolase, triose isomerase, α -glycerophosphate dehydrogenase, phosphoglycerate kinase, glyceraldehyde phosphate dehydrogenase, hexokinase and pyruvic phosphokinase.

The co-enzymes DPNH, ATP and ADP were obtained from the same source.

Estimation of Metabolites.

Inorganic phosphate was estimated by the method of Fiske and Subbarow (1925). Total phosphate (inorganic and bound phosphate) estimations were made by boiling the sample for 5 minutes with 1 ml. of 60% HClO_4 and then cooling. The sample was then treated as for inorganic phosphate estimations.

The metabolites, pyruvate, α -ketoglutarate, fructose diphosphate and adenosine triphosphate (ATP) were estimated by standard enzymic methods described in the technical bulletins of C. F. Boehringer und Soehne, Mannheim, West Germany.

COMPARISON OF THE ACTION OF CANTHARIDIN ON LIVING AND DEAD EPITHELIUM.

Although it seemed likely that cantharidin was acting on some reaction in the living cell, it was considered important to demonstrate that the action of cantharidin was dependent on the presence of vital mechanisms.

A fresh specimen of normal skin was stripped of fat. The corneus layer was removed by stripping with Sellotape fifty times. The remaining full thickness skin less the horny layer was placed in liquor epispasticus at room temperature. After 24 hours, the specimen was fixed, cut and stained with haematoxylin and eosin. The cells of the decornified epithelium showed normal intercellular bridges.

In a second experiment, fresh normal skin was stripped of fat. The epithelium was removed by holding the skin tightly stretched over a hot plate at 65°C . This epithelium was placed in liquor epispasticus at room temperature and a control piece in normal saline. These were removed after 24 hours and fixed, cut and stained. The epithelial structure was preserved in both specimens.

From these experiments, it was concluded that cantharidin produced its epithelial damage by some action on a living system rather than by a simple chemical destructive process. The fact that vital mechanisms were essential for the acantholytic process to occur suggested that an analysis of the action of cantharidin and DSC on enzyme systems might provide some indication of the mode of action of these toxic substances. It was decided to re-investigate the effect of cantharidin on tissue slice metabolism.

THE EFFECT OF CANTHARIDIN ON RESPIRATION AND GLYCOLYSIS IN VARIOUS TYPES OF ANIMAL CELLS.

The skin itself is not very satisfactory for respiratory experiments. This is largely due to the relatively low metabolic rate of adult skin as compared with other tissues. Also, Guzman Barron *et al.* (1948) have commented on the variability of the normal rates of respiration of skin.

In view of this, the action of cantharidin has been studied on the respiration of other tissues.

Respiration of Rat Liver Slices.

Rat liver slices (approximate wet weight = 50 mg.) were suspended in Krebs-Ringer phosphate saline ($\pm$ cantharidin, saturated solution at 37° C., final concentration approximately 0.0001 M) in an atmosphere of O₂. The O₂ uptake was measured, the CO₂ being absorbed with KOH papers. The total volume of the system was 2.0 ml.

The figures (Table I) refer to to μ l. O₂/50 mg. wet weight of liver.

TABLE I.—*The effect of cantharidin on the respiration of rat liver slices.*

System	Period of incubation.	
	First hour.	Second hour.
Control	66	53
Cantharidin	49	34
% Inhibition	25.8	35.8

Inhibition of respiration was observed during the first and second hours of incubation at 37° C. in the presence of cantharidin.

Respiration of Rat Brain Cortex Slices.

Rat brain cortex slices (approximate wet weight = 80 mg.) were suspended in Krebs-Ringer phosphate saline ($\pm$ cantharidin, saturated solution at 37° C., final concentration approximately 0.0001 M) with glucose 0.1 M in an atmosphere of O₂. The O₂ uptake was measured, the CO₂ being absorbed with KOH papers. The total volume of the system was 2.0 ml.

The figures in Table II refer to μ l. O₂/100 mg. wet weight of rat brain cortex.

TABLE II.—*The effect of cantharidin on the respiration of rat brain cortex slices.*

System	Period of incubation.	
	First hour.	Second hour.
Control	133	135
Cantharidin	116	99
% Inhibition	12.8	26.7

As with rat liver slices, cantharidin inhibited the rate of oxygen uptake. This might have been due to a reduction in terminal oxidative mechanisms leading to a concurrent accumulation of keto acids. This possibility was tested by estimating keto acids in the incubation systems (brain slices $\pm$ cantharidin after 2 hours at 37° C.). Both systems contained 0.095 μ moles of pyruvate and neither contained α -ketoglutarate, suggesting that cantharidin had no appreciable effect on lactic dehydrogenase, glutamic acid dehydrogenase, pyruvic oxidase or α -ketoglutarate dehydrogenase.

The observed inhibition of cellular respiration by cantharidin, although significant, was not very great. Its effect on glycolysis was therefore examined.

Anaerobic Glycolysis of Ehrlich Ascites Tumour Cells.

Ascites tumour cells characteristically exhibit a high rate of anaerobic glycolysis. Ascites tumour cells (approximately 25 mg.) were suspended in Krebs-Ringer bicarbonate saline and glucose 0.005 M, one flask containing a saturated solution of cantharidin at 37° C. (final concentration approximately 0.0001 M). Additional flasks contained DSC in concentrations of 0.004 M and 0.0008 M. These were incubated in 95% N₂ and 5% CO₂ and the CO₂ output measured. The total volume of the system was 2.7 ml.

The figures in Table III refer to μ l. CO₂/25 mg. wet weight of ascites tumour cells.

TABLE III.—*Comparison of the effect of cantharidin and DSC on anaerobic glycolysis of Ehrlich ascites tumour cells.*

System	60 minutes.	% Inhibition at 60 minutes.	120 minutes.	% Inhibition at 120 minutes.
Control	105	—	95	—
Cantharidin 0.0001 M	84	20	56	41
DSC 0.004 M	60	43	44	52.6
DSC 0.0008 M	76	27.8	52	45

After a lag period, cantharidin markedly inhibited the rate of anaerobic glycolysis by these cells and water soluble DSC produced a comparable degree of inhibition.

THE EFFECT OF DSC ON YEAST METABOLISM.

Following the demonstration that cantharidin and DSC inhibited both respiration and glycolysis in various types of animal cells, the effect of the inhibitor on yeast metabolism was examined. Yeast was chosen for study because it possesses glycolytic properties which may relatively easily be studied in cell-free systems and the effect of cantharidin and DSC on animal tissues, although significant, is low on glycolysis and particularly on respiration. Accordingly yeast cell suspensions, if affected by DSC, might be expected to give more consistently reproducible results.

Yeast cells (approximately 6 mg.) were suspended in KH₂PO₄ 0.04 M and glucose 0.05 M ($\pm$ DSC, final concentration 0.0033 M) in an atmosphere of 95% N₂ and 5% CO₂ at 37° C. The total volume of the system was 3.5 ml.

The figures in Table IV refer to μ l. CO₂/6 mg. of fresh weight baker's yeast.

TABLE IV.—*The effect of DSC on the anaerobic glycolysis of baker's yeast.*

System	Period of incubation.	
	30 minutes.	60 minutes.
Control	370	280
DSC	275	125
% Inhibition	25	55

The effect of DSC on the anaerobic glycolysis of baker's yeast was found to be comparable in degree to its effect on the anaerobic glycolysis of ascites

tumour cells. However the effect of DSC on the aerobic respiration of baker's yeast in the presence of glucose was to produce a more marked inhibition of respiration than that of cantharidin on animal tissues.

Yeast cells (approximately 2.5 mg.) were suspended in KH_2PO_4 0.01 M and glucose 0.05 M in an atmosphere of O_2 at 37°C . Apart from the control, flasks contained DSC in concentrations of 0.01 M, 0.001 M, 0.0001 M and 0.00001 M. The total volume of the system was 2 ml., CO_2 being absorbed with KOH papers.

The figures on Fig. 2 refer to $\mu\text{l. O}_2/2.5 \text{ mg. of baker's yeast}$.

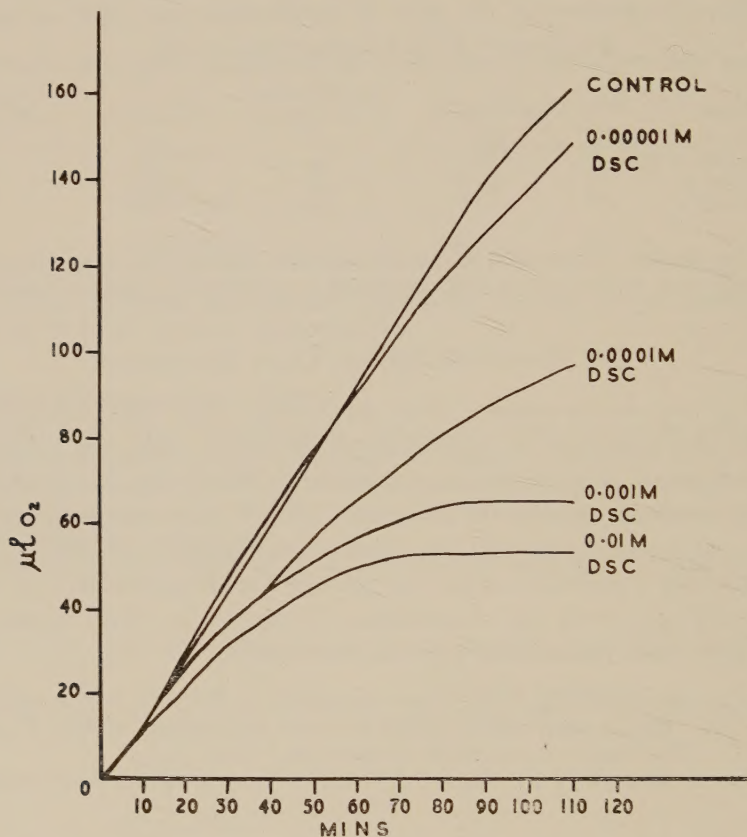


FIG. 2.

The graph shows that the higher the concentration of DSC, the greater was the inhibition of yeast respiration, with no evidence of activation at the lower concentrations.

On the other hand, when glucose was omitted from the media, the presence of DSC was found to be associated with an increased oxygen uptake by whole yeast cells.

Yeast cells (50 mg.) were incubated in KH_2PO_4 0.01 M ($\pm$ DSC, final concentration 0.004 M) in air at 37°C ., the CO_2 being absorbed with KOH papers. The total volume of the system was 2.5 ml.

The figures in Fig. 3 refer to $\mu\text{l. O}_2/50\text{ mg. of baker's yeast}$.

The arrow indicates the time of addition of the DSC.

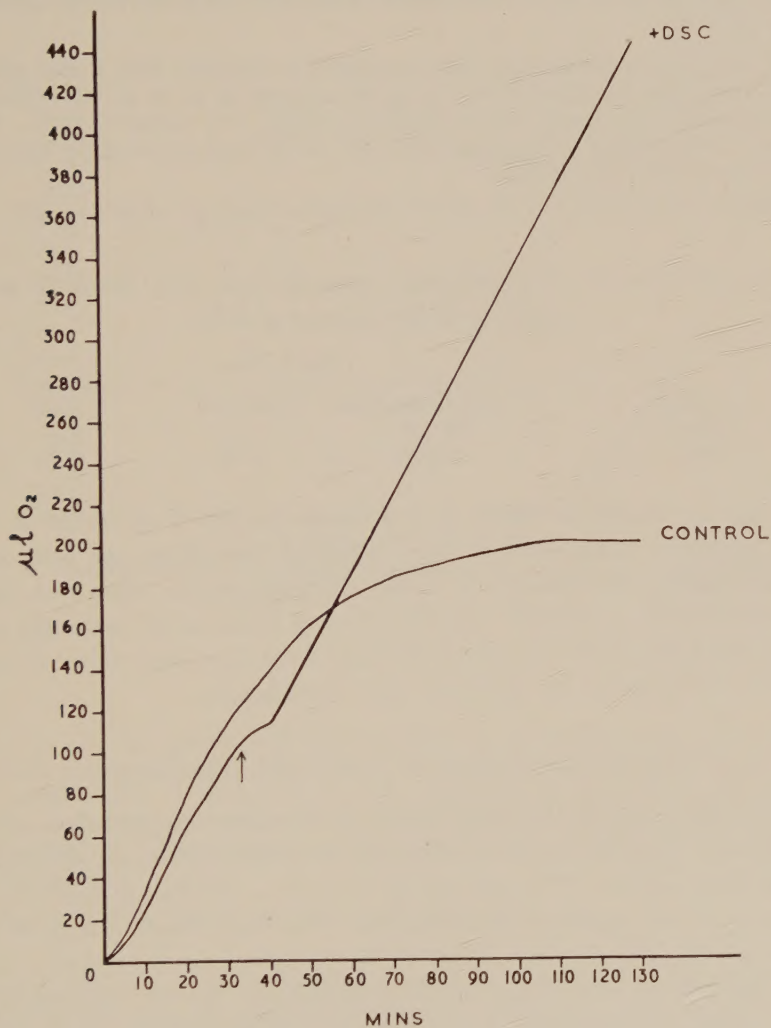


FIG. 3.

This surprising result could be interpreted as being due either to DSC being oxidized by yeast cells or to DSC having accelerated the endogenous respiration of intact yeast. Uncoupling agents often increase the rate of endogenous respiration due, possibly, to the non-utilization of substrates and energy for biosynthesis and a similar effect may be considered here. Uncoupling agents

have been postulated to inhibit the resynthesis of ATP from ADP and phosphate (Hunter, 1951). This aspect of the problem was not pursued further.

To obtain a possible lead on the manner in which DSC might be inhibiting yeast cell respiration, the concentrations of the three metabolites fructose 1 : 6 diphosphate, pyruvic acid and ATP were measured in yeast cells respiring in the presence and absence of DSC.

Yeast cells (4 g. fresh weight) were incubated in KH_2PO_4 0.01 M and glucose 1 M ($\pm$ DSC, final concentration 0.005 M) for 90 minutes in O_2 at 37°C . After centrifuging, the cells were lysed with ice-cold 5% HClO_4 and cooled to 0°C . Insoluble KClO_4 was precipitated by titration with 20% KOH and removed by filtration, the filtrate represented cell contents.

The figures in Table V refer to μmoles of metabolites/4 g. baker's yeast.

TABLE V.—*Fructose 1 : 6 di-phosphate, pyruvate and ATP levels in yeast cells respiring in the presence of DSC.*

System	Metabolites.		
	Fr. 1 : 6 diphosphate.	Pyruvate.	ATP.
Control . . .	0.045	1.17	3.04
DSC	0.050	0.98	0.77

DSC had no significant effect on the concentration of pyruvate or fructose 1 : 6 diphosphate, from which it was concluded that there had been no significant inhibition of aldolase or lactic dehydrogenase. However the concentration of ATP in the DSC-treated cells was found to be strikingly reduced. Such an effect on ATP concentration could have been due either to impaired synthesis of ATP or to an increased rate of utilization.

THE EFFECT OF DSC ON CELL-FREE PREPARATIONS.

In view of the finding that DSC inhibited the anaerobic glycolysis of baker's yeast, it was decided to study its effect on anaerobic glycolysis, brought about by a cell-free extract. The advantage of such a cell-free preparation lay in the absence of any permeability barriers. The chief disadvantage of such an extract was that by the very nature of the complex enzyme systems involved, it was difficult to obtain an active preparation. In all experiments with Lebedev juice, acetaldehyde was added to the medium for the purpose of converting the DPNH in the Lebedev juice to the oxidized DPN. This was brought about by alcohol dehydrogenase, with the formation of DPN and alcohol.

Glucose (100 mg.) was incubated in phosphate buffer 0.01 M, pH 7.4, with Lebedev juice (1 ml.) and 2% acetaldehyde (1 drop) in air at 28°C . ($\pm$ DSC, final concentration 0.0083 M), the total volume of the system being 2 ml.

The figures in Fig. 4 refer to $\mu\text{l. CO}_2$ /1 ml. Lebedev juice.

Anaerobic glycolysis by Lebedev juice was found to start only after a considerable lag period, in the region of 50 minutes. The effect of DSC was to prolong this lag period for a further 30 minutes.

The effects of ATP and DPN on this lag period were studied and were compared with those of DSC and cantharidin.

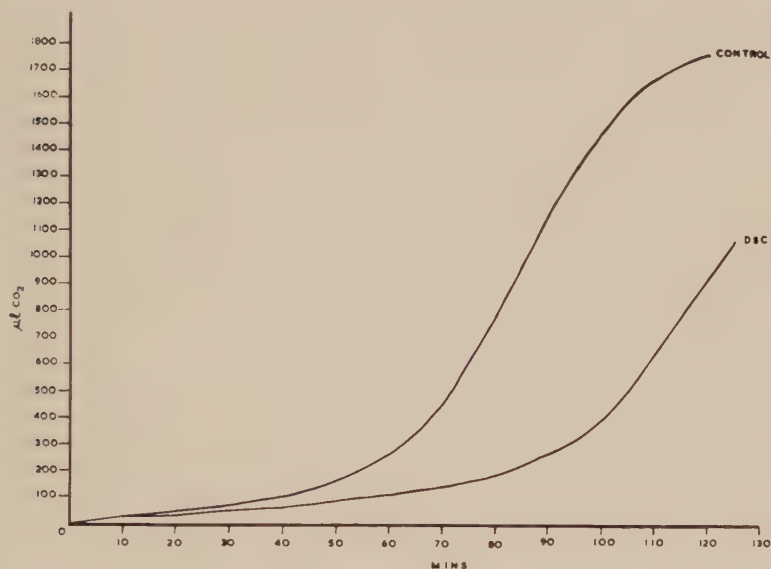


FIG. 4.

In each of 5 flasks glucose (100 mg) was placed in phosphate buffer 0.02 M, pH 7.4, with Lebedev juice (1 ml.). To each flask the following additions were made (1) + 2% acetaldehyde (1 drop), (2) + 2% acetaldehyde (1 drop) + DSC 0.005 M, (3) + 2% acetaldehyde (1 drop) + cantharidin 0.0001 M, (4) + ATP 0.01 M, (5) + DPN 0.0007 M. These were incubated in air at 28° C., the total volume of the system being 2 ml.

The figures in Fig. 5 refer to $\mu\text{l. CO}_2/\text{1 ml. Lebedev juice}$.

It was found that the lag period was shortened by the addition of DPN and more so by that of ATP, in comparison with the prolongation produced by both DSC and cantharidin. It was then shown that the effect of ATP was to reduce the increase in the lag period brought about by DSC.

Glucose (100 mg.) and Lebedev juice (1 ml.) were incubated in phosphate buffer 0.01 M, pH 7.4, with 2% acetaldehyde (1 drop) and ATP 0.016 M ($\pm$ DSC, final concentration 0.004 M) in air at 28° C., the total volume of the system being 1.2 ml.

The figures in Fig. 6 refer to $\mu\text{l CO}_2/\text{1 ml. Lebedev juice}$. The arrow indicates the time of addition of ATP.

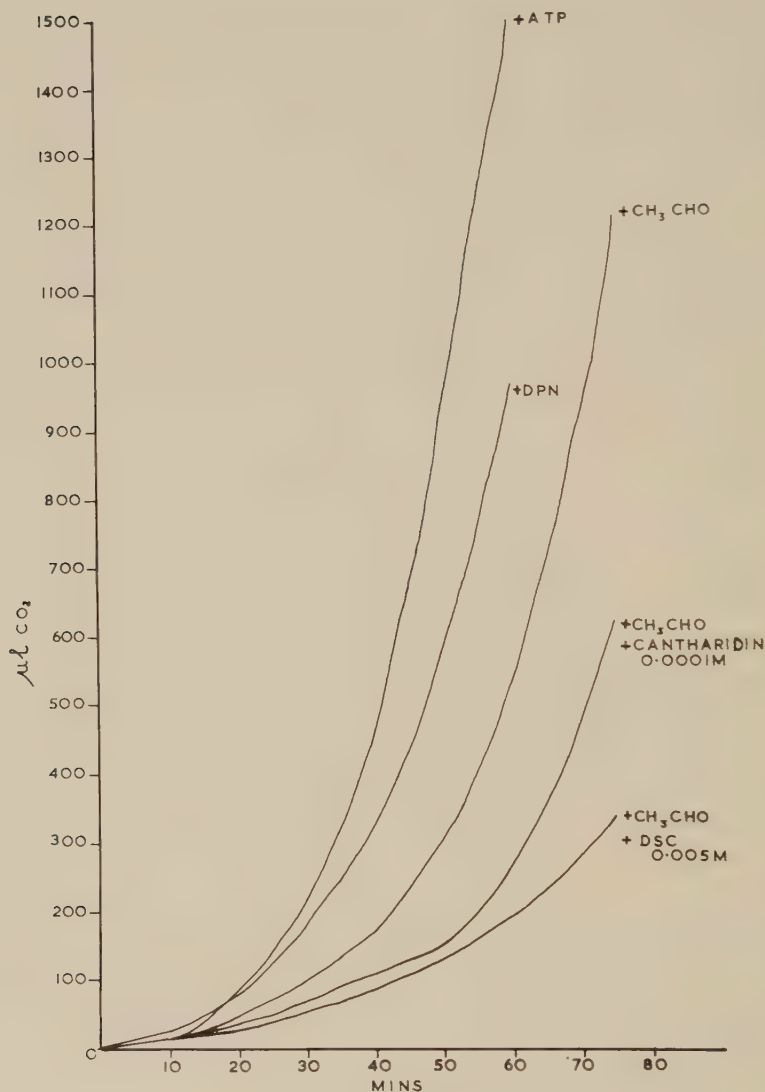


FIG. 5.

This lent further support to the possibility that the action of DSC was either to inhibit the regeneration of ATP or to increase its rate of utilization.

EFFECT OF DSC ON ISOLATED ENZYMES.

Although both glycolysis and respiration in animal and yeast cells were inhibited, the effect of the inhibitor on the glycolytic sequence was selected for detailed analysis because of the comparative ease of obtaining the individual enzymes in this system.

Effect on Hexokinase.

As hexokinase, which phosphorylates glucose to glucose 6-phosphate in the presence of ATP and Mg^{++} , is the pace-making step in the glycolytic system, it was of interest to test the action of DSC on purified yeast hexokinase.

The rate of phosphorylation was measured manometrically by the CO_2 liberated from a bicarbonate medium, by the newly formed acid radicals of the ADP and glucose 6-phosphate.

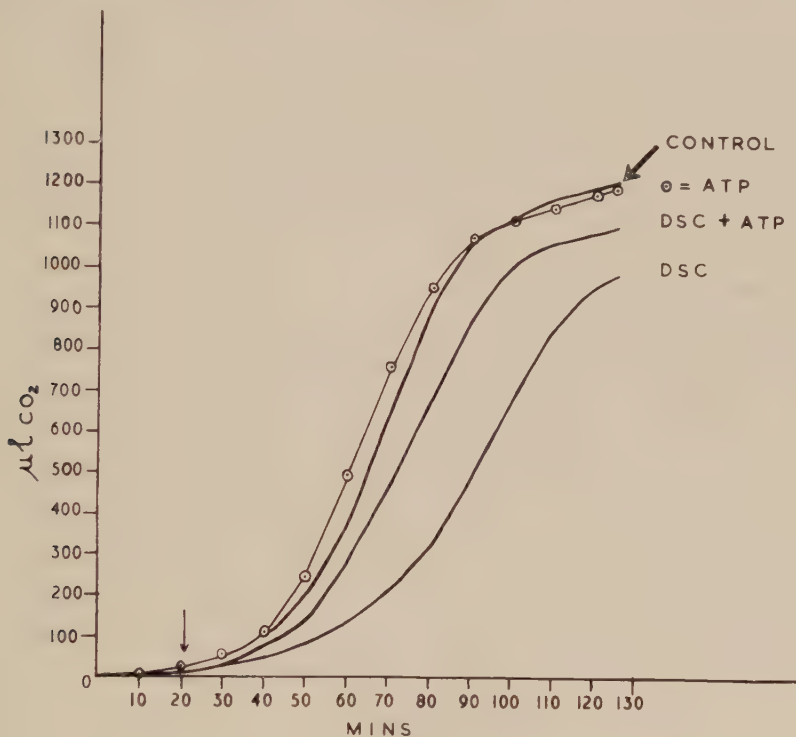


FIG. 6.

Hexokinase was incubated in sodium bicarbonate, with glucose, sodium fluoride, $MgCl_2$ and ATP ($\pm$ DSC and cantharidin) in CO_2 and N_2 at $37^\circ C$.

In the light of later findings it is important to stress that Mg^{++} were present in excess, under these conditions no inhibition of yeast hexokinase was detected.

Effect on Phosphohexokinase.

Phosphohexokinase, in the presence of ATP and Mg^{++} , phosphorylates fructose 6-phosphate to fructose 1 : 6 diphosphate.

Phosphohexokinase was incubated in a similar system to that used for hexokinase.

No inhibition of phosphohexokinase was detected: again it must be pointed out that Mg^{++} were added in excess for this experiment.

Effect on Triose Phosphate Dehydrogenase System.

Inhibition of aldolase by DSC has been shown to be unlikely, as no abnormal accumulation of fructose 1 : 6 diphosphate could be detected in yeast cells respiring

in the presence of DSC (Table V). The next step in the glycolytic pathway to be investigated was that from glyceraldehyde 3-phosphate to 1 : 3 diphosphoglyceric acid, a step catalyzed by glyceraldehyde phosphate dehydrogenase.

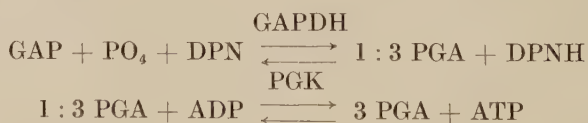
The method used was that described by Roitt (1956). Triose phosphate formed by the action of aldolase on fructose diphosphate was oxidized by glyceraldehyde phosphate dehydrogenase and DPN in the presence of arsenate to 1-arseno-3-phosphoglyceric acid. At the same time pyruvate in the presence of lactic acid dehydrogenase reoxidized DPNH. The 3-phosphoglyceric acid, formed by the spontaneous hydrolysis of 1-arseno-3-phosphoglyceric acid was estimated manometrically by the CO_2 which it liberated from the bicarbonate in the medium, thereby giving a measure of the aldolase, glyceraldehyde phosphate dehydrogenase and lactic dehydrogenase activity in the system.

Dialyzed rabbit muscle extract was used as the source of the enzymes. No inhibition of the triose phosphate dehydrogenase system could be detected. From this it was concluded that none of the enzymes taking part in this system, aldolase, glyceraldehyde phosphate dehydrogenase or lactic dehydrogenase, were inhibited by DSC.

Effect on Phosphoglyceric Kinase.

The results of the investigations into the effect of DSC on yeast phosphate metabolism and on cell-free glycolytic preparations suggested the possibility that the action of DSC may be to inhibit a system responsible for the regeneration of ATP. The two enzymes in the glycolytic pathway responsible for the regeneration of ATP, phosphoglyceric kinase and pyruvic phosphokinase were therefore tested.

Phosphoglyceric kinase (PGK) in the presence of Mg^{++} , transports high energy phosphate from 1 : 3 diphosphoglyceric acid (1 : 3 PGA) to ADP with the subsequent formation of ATP and 3-phosphoglyceric acid (3 PGA). Phosphoglyceric kinase activity was measured by coupling this enzyme with glyceraldehyde phosphate dehydrogenase (GAPDH) and DPNH, according to the equation given below. The presence of cysteine was necessary to ensure that the reaction proceeded to the left in favour of the accumulation of glyceraldehyde phosphate (GAP) and DPN, a change which could be measured spectrophotometrically.



This experiment was initially performed in the presence of an excess of Mg^{++} , in the form of MgCl_2 (final concentration 0.003 M). In these circumstances no inhibition of phosphoglyceric kinase was detected. It appeared possible that the action of DSC might be to compete for Mg^{++} . The experiment was therefore repeated without the addition of magnesium.

Phosphoglyceric kinase 30 $\mu\text{g.}$ was incubated with ATP 0.003 M for 10 minutes at 37°C. in Tris buffer 0.03 M, pH 7.6, before being added to DPNH 500 $\mu\text{g.}$, glyceraldehyde phosphate dehydrogenase 200 $\mu\text{g.}$, cysteine 0.003 M ($\pm$ DSC, final concentration 0.0015 M).

The time in Fig. 7 refers to minutes following the addition of phosphoglyceric acid.

In these circumstances, with no added magnesium, DSC was found to produce a 30% inhibition of phosphoglyceric kinase.

Effect on Pyruvic phosphokinase.

The second mechanism responsible for the regeneration of ATP during glycolysis remained to be tested. Pyruvic phosphokinase (PPK), in the presence of ADP and Mg^{++} catalyzes the breakdown of phospho-enol pyruvic acid (PEP) to pyruvic acid (PA) with the formation of ATP.

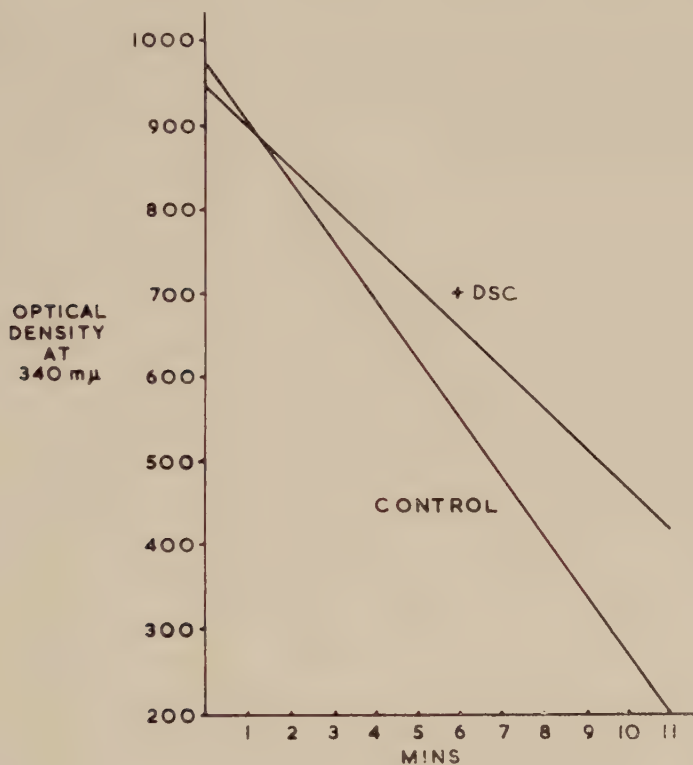


FIG. 7.

Pyruvic phosphokinase was assayed by following the formation of pyruvic acid with lactic dehydrogenase (LDH) and DPNH according to the following equation. Lactic acid is represented by LA.



The change from DPNH to DPN was measured spectrophotometrically.

In view of the findings with phosphoglyceric kinase, the first experiment with pyruvic phosphokinase was performed using no additional Mg^{++} .

Phospho-enol pyruvate 0.0015 M was added to Tris buffer 0.08 M, pH 7.6, ADP 0.0015 M, pyruvic phosphokinase 5 $\mu\text{g.}$, DPNH 0.00024 M and lactic dehydrogenase 100 $\mu\text{g.}$ ($\pm$ DSC, final concentration 0.0015 M).

In Fig. 8, the arrow indicates the time of addition of pyruvate to give a final concentration of 0.00008 M.

Fig. 8 shows the inhibitory effect of DSC on this system. This might have been due either to the effect of DSC on the pyruvic phosphokinase or on the lactic dehydrogenase in the system. Evidence has already been put forward that lactic dehydrogenase is not inhibited. This was confirmed in the present experiment by adding pyruvate (final concentration 0.00008 M) at 16 minutes. The curve clearly shows the sudden fall in optical density of the test solution following this procedure, indicating that the enzymic block did not effect the step from pyruvic acid $\rightarrow$ lactic acid, thus confirming that the inhibitory effect of DSC was being exerted on the pyruvic phosphokinase in the system.

This experiment was then repeated with the addition of excess Mg^{++} , giving a final concentration of MgCl_2 0.003 M . With this addition, no inhibition of pyruvate phosphokinase could be detected.

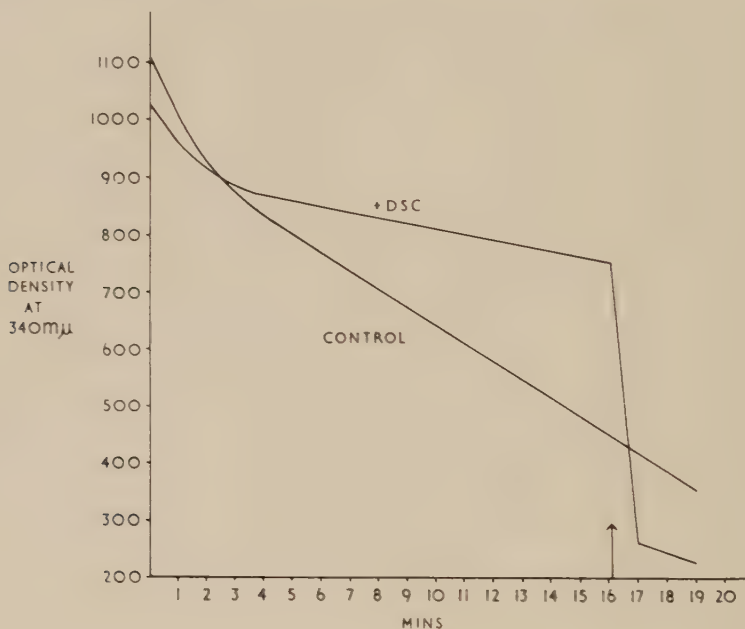


FIG. 8.

Finally the experiment was repeated with a concentration of Mg^{++} (0.00065 M), which was insufficient to give full activity in this system, but gave a measurable steady rate of reaction.

Phospho-enol pyruvate 0.0015 M was added to Tris buffer 0.08 M , pH 7.6, ADP 0.0015 M , pyruvic phosphokinase $20\text{ }\mu\text{g.}$, DPNH 0.0002 M and lactic dehydrogenase $100\text{ }\mu\text{g.}$ ($\pm$ DSC, final concentration 0.0015 M).

In Fig. 9, the first arrow indicates the start of the reaction by the addition of phospho-enol pyruvate and the two subsequent arrows indicate the time of addition of MgCl_2 to give final concentrations of 0.00065 M and 0.0016 M .

It can be seen from the graph (Fig. 9) that with this concentration of Mg^{++} , there was a 66% inhibition by DSC. On adding more Mg^{++} , to give a final concentration of 0.0016 M , this inhibition was shown to be reversed.

From these results, it was concluded that the inhibitory action of DSC on the glycolytic pathway was intimately related to the requirements of certain steps of this pathway for magnesium.

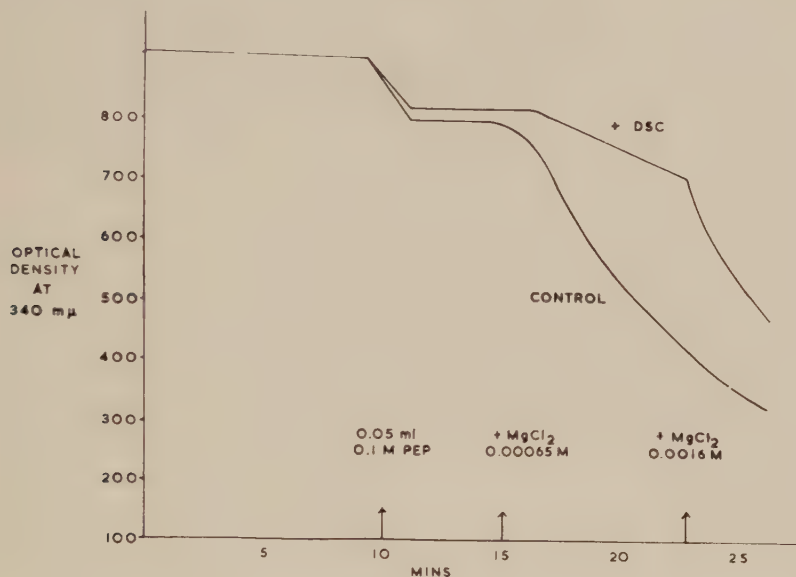


FIG. 9.

DISCUSSION.

Although these biochemical studies constitute only a cursory examination of what appears to be a very complex problem, certain significant findings have been obtained experimentally, which may indicate the mechanism of the toxic action of cantharidin.

Water soluble disodium cantharidin was prepared by treating cantharidin with two molecular equivalents of sodium hydroxide. It was shown that this compound had the same vesicant properties in high dilution as fat soluble cantharidin. It was therefore used for the majority of the enzyme experiments, its solubility in water making it more suitable for this work than cantharidin itself. Following the observation that disodium cantharidin inhibited glycolysis in ascites tumour cells, its effect was examined on yeast cells, which if inhibited would be expected to give more reproducible results in the search for a specific point of action of an inhibitor of glycolysis. Inhibition of yeast glycolysis and respiration was shown to occur and these cells were therefore used to study the effect of disodium cantharidin on the levels of cellular intermediates of metabolism. This part of the work demonstrated a striking fall in the level of ATP in the cells respiring in the presence of disodium cantharidin.

These disconnected observations gained some meaning from the results of the experimental studies on isolated enzyme systems. Of the enzymes tested, only two were found to be significantly inhibited. These both required magnesium ions. Of the inhibition of phosphoglyceric kinase and pyruvic phosphokinase, the latter was the more pronounced and this inhibition proved reversible by magnesium ions. Since magnesium ions are required for many reactions and disodium cantharidin inhibition is reversed by them, it was possible that the effect of the inhibitor might be on ion binding by enzymes requiring magnesium for activation.

Such an explanation would be supported by the findings described. ATP production and utilization each require magnesium ions and accordingly, biosynthesis is largely dependent on magnesium as is the trapping of energy for oxidation and glycolysis of substrates. The non-utilization of magnesium would be expected to cause impaired formation of ATP and an uncoupling of energy production and inhibition of many biosynthetic sequences. A tissue deprived of magnesium ions would be expected to show imbalance between catabolic and anabolic processes with a net increase in proteolysis (Schoenheimer, 1949).

Stoughton (1957) has suggested that the actual process of blistering by cantharidin may be preceded by the liberation of proteolytic enzymes. It is probable, however, on the basis of Schoenheimer's concept of the dynamic state of living tissues, that there is no *release* of a proteolytic enzyme but an apparent net increase in proteolytic activity within the tissue.

If the observed increased oxygen uptake by yeast cells respiring endogenously in the presence of disodium cantharidin is due to uncoupling of energy production, the increased oxygen uptake would be expected since resynthesis of carbohydrates would be prevented in the absence of ATP.

Whether disodium cantharidin inhibits magnesium ion binding by enzymes in general, or only a limited number, was not determined. Of the 660 enzymes listed by Dixon and Webb (1958) approximately 70 require magnesium ions for activation. It is therefore apparent that testing the specificity of the disodium cantharidin action on all enzymes requiring magnesium ions would necessitate a prolonged investigation.

The fact that disodium cantharidin appears to inhibit the utilization of magnesium ions, together with the observation of Allison and Bettley (1958) that the normal skin of subjects with active atopic eczema reacts excessively to the vesicant action of cantharidin, would appear to be in agreement with the observations of Engman and McCardle (1940, 1941 and 1942) on the magnesium content of the skin of atopic subjects. They found a marked deficiency of magnesium ions in the cutaneous lesions of these subjects and subnormal values in the normal uninvolved skin in this disease. Also, it has been shown experimentally in the rat (Sullivan and Evans, 1944

and 1945) that magnesium deficiency may result in an eczematous type of reaction.

The suggestion is therefore put forward that the increased blistering reaction to cantharidin found in subjects with atopic eczema may be explained by the fact that their skin, being already deficient in magnesium ions, it is more easily damaged by an agent that is capable of inhibiting the utilization of magnesium ions for metabolic purposes.

SUMMARY.

The acantholytic action of cantharidin was shown to be dependent on vital mechanisms.

The preparation of water-soluble disodium cantharidin is described. It was shown to have the same vesicant properties as fat soluble cantharidin.

The respiration and anaerobic glycolysis of mammalian tissue and yeast cells were inhibited by cantharidin and disodium cantharidin. On the other hand, the oxygen uptake of yeast cells, respiring in the absence of glucose, was increased by the presence of disodium cantharidin. A fall in the intracellular ATP concentration was shown by yeast cells respiring in the presence of disodium cantharidin. The lag period in the onset of glycolysis by a cell-free yeast extract was prolonged by disodium cantharidin. This effect is partially counteracted by ATP.

Evidence is presented that the following enzymes were not inhibited by disodium cantharidin: aldolase, glyceraldehyde phosphate dehydrogenase, lactic dehydrogenase, pyruvic oxidase and α -ketoglutarate dehydrogenase. Hexokinase and phosphohexokinase were not inhibited in the presence of excess magnesium ions.

The two glycolytic, ATP regenerating enzymes, phosphoglyceric kinase and pyruvate phosphokinase were inhibited by disodium cantharidin in the absence of excess magnesium ions. This inhibition was reversed by the addition of magnesium ions.

The implications of this apparent inhibition of the utilization of magnesium ions are correlated with the finding of an increased vesicant response to cantharidin, previously described in subjects with atopic eczema.

We wish to thank Professor F. Dickens, F.R.S., for his advice and for facilities for carrying out this work in his department at the S. A. Courtauld Institute of Biochemistry, the Middlesex Hospital, Dr. R. D. Batt for advice on the preparation of the paper, Mr. H. E. H. Jones for performing the animal experiments and Dr. J. G. Bennette for the ascites tumour cells.

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VITAMIN C AND EXFOLIATIVE DERMATITIS.

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As an extension of our researches into exfoliative dermatitis, we decided to investigate some aspects of vitamin C metabolism in this condition. Accordingly, ascorbic acid saturation tests were carried out, together with estimations of blood vitamin C. Some abnormalities were observed which will be described in detail below. Since patients with scurvy excrete greatly increased amounts of tyrosyl derivatives when given large quantities of tyrosine by mouth, measurements of tyrosyl derivatives were also made in four normal people and six patients with exfoliative dermatitis.

INVESTIGATION.

(i) Vitamin C saturation tests were carried out as follows. A dose of 70 mg. ascorbic acid for every stone body weight (i.e. 11 mg. kg.) was given to the subject by mouth. Four hours later, the bladder was emptied and the urine thrown away. All urine passed during the succeeding two hours was collected and the vitamin C content measured by the method of Harris and Ray (1935). This procedure was repeated daily until a urinary excretion of 50 mg. ascorbic acid in 2 hours was attained.

The subjects in whom this test was performed are listed in Table I.

TABLE I.—*Cases in which Vitamin C Saturation Tests were Performed.*

Condition.	Number of Cases.
Normal	25
Exfoliative dermatitis following	
Eczema	15
Psoriasis	4
Reticulosis	3
Pemphigus foliaceus	2
Drugs	2
Eczema	12
Psoriasis	13
Varicose eczema	4
Varicose ulcers	4

(ii) Vitamin C saturation tests with concurrent blood vitamin C levels were carried out in two normal subjects and ten cases of exfoliative dermatitis. Whole blood ascorbic acid was measured by the method of Roe and Kuether (1945).

(iii) Experiments with tyrosine were conducted in the following way. Twenty-four hour samples of urine were collected for 11 consecutive days. On days 1-3 no tyrosine was administered. On days 4-8 inclusive, 20 g. of L-tyrosine powder were given daily in 4 doses of 5 g. each. On days 9-11, no tyrosine was given. Every day duplicate determinations were made of the concentration of tyrosyl derivatives in samples of urine using a modification of Millon's test as described by Medes (1932).

These tests were performed in four normal subjects and in six cases of exfoliative dermatitis.

TABLE II.—*Number of Days Required for Saturation with Vitamin C.*

	Normal.	Exfoliative dermatitis following					Drugs.	Eczema.	Psoriasis.	Varicose eczema.	Varicose ulcer.
		Eczema.	Psoriasis.	Reticulosis.	P. foliaceus.						
Mean	1.7	10.4	7.8	7.3	4.5	1.6	4.1	4.0	4.8	2.3	
Standard deviation and series	0.84	6.09	3.11	1.23	1.0	2.93	2.58	1.77	2.08	0.56	
$P < .$	—	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.6	

P = probability (Student's " t "-test) that the observed difference between the normal and the particular pathological group arises by chance. All of the differences in this table are significant except for varicose ulcer.

RESULTS.

Table II shows the results of ascorbic acid saturation tests in the various groups. As can be seen, patients with any of the skin diseases studied, except varicose ulcer, took longer to saturate with vitamin C than normal people. Furthermore, patients with exfoliative dermatitis took significantly longer to become saturated than patients with less extensive involvement with the disease from which the exfoliative dermatitis derived; the mean number of days required to saturate patients with exfoliative dermatitis following eczema (10.4 days) was significantly greater ($P < 0.01$) than the mean number of days required for patients with eczema (4.1 days). Similarly the group with exfoliative dermatitis following psoriasis needed a significantly greater time (7.82 days) for saturation than the group with psoriasis only (4.0 days; $P < 0.01$).

The concurrent blood ascorbic acid and ascorbic acid saturation tests were performed to ensure that the abnormal saturation tests observed in exfoliative dermatitis were not due to failure of absorption of vitamin C. Since only two normal volunteers were willing to undergo daily venepuncture for some weeks, statistically significant results can barely be expected. Table III gives figures for blood ascorbic acid in mg.%, before treatment with vitamin C, when urinary ascorbic acid began to rise and when urinary excretion reached 50 mg./2 hours. Although statistical analysis is impossible, it seems probable that the initial level is low in exfoliative dermatitis.

TABLE III.—*Blood Ascorbic Acid in mg.% during Treatment with Large Doses of Ascorbic Acid.*

	Blood ascorbic acid.		
	Before treatment.	When urinary ascorbic acid rose.	When urinary ascorbic acid reached 50 mg./2 hr.
Normal subjects . . .	1.3	1.3	1.5
	1.0	1.4	2.1
Exfoliative subjects . . .	0.6	1.1	2.0
	0.4	1.3	2.3
	1.0	2.0	2.5
	0.9	1.1	2.2
	0.8	1.7	2.7
	0.3	1.6	1.8
	0.5	1.3	1.9
	0.4	1.5	2.2
	0.6	1.6	2.5
	0.6	1.0	2.7

Fig. 1 shows the typical response to ascorbic acid of normal subjects and patients with exfoliative dermatitis. Since the blood ascorbic acid of exfoliatives began to rise as soon as vitamin C was given, it appears likely that abnormalities of the vitamin C saturation test in these cases are not due to failure of absorption.

FIG 1. BLOOD CONCENTRATION AND URINARY EXCRETION OF ASCORBIC ACID DURING ADMINISTRATION OF VITAMIN C.

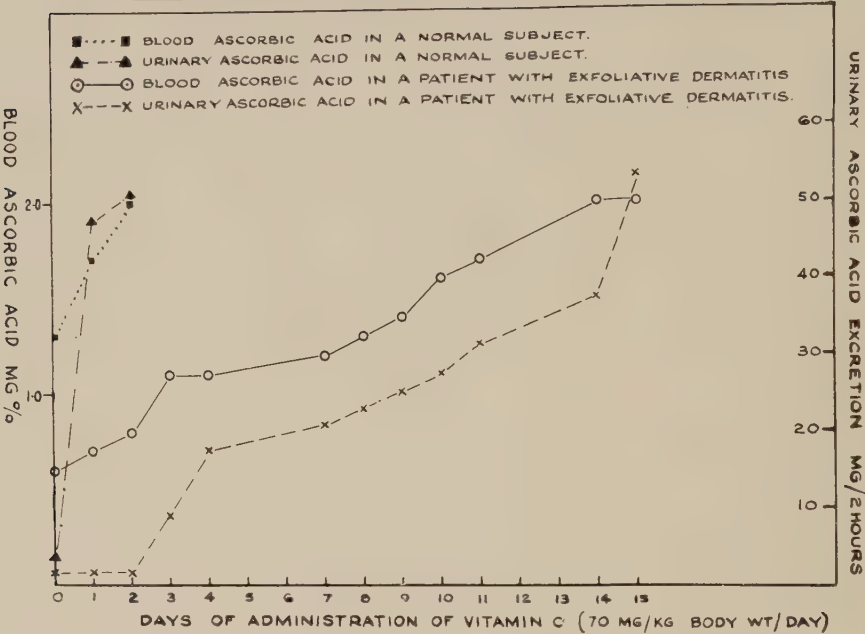
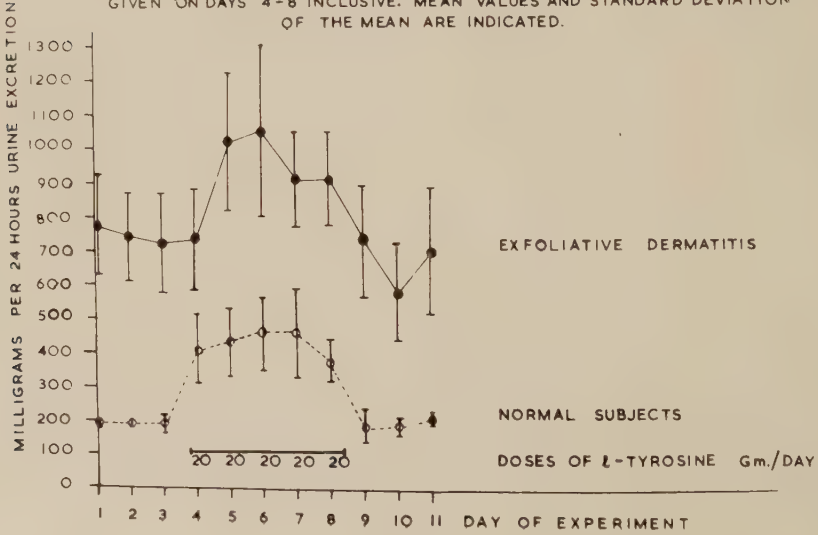


FIG.2 EXCRETION OF TYROSYL DERIVATIVES IN URINE BY NORMAL SUBJECTS AND PATIENTS WITH EXFOLIATIVE DERMATITIS. 20GM. L-TYROSINE POWDER/DAY GIVEN ON DAYS 4-8 INCLUSIVE. MEAN VALUES AND STANDARD DEVIATION OF THE MEAN ARE INDICATED.



The excretion of tyrosyl derivatives is shown in Fig. 2. The excretion of tyrosyl derivatives was significantly greater in cases of exfoliative dermatitis both before and after administration of tyrosine. Nevertheless, the response to added tyrosine was the same in both groups and except for the higher level throughout in the exfoliatives, both groups have substantially the same form.

DISCUSSION.

We have shown that cases of exfoliative dermatitis took longer to saturate with vitamin C than normal subjects. This is not specific for exfoliative dermatitis as cases of eczema, psoriasis and varicose eczema also took an abnormally long time to saturate. It seemed, moreover, that the time taken for saturation was proportional to the extent of the skin disease, since cases of exfoliative dermatitis following eczema took a mean of 10.4 days while the cases of simple eczema took 4.1 days. Similarly patients with psoriatic erythroderma took 7.8 days on the average for saturation in contrast to the mean of 4.0 days required for simple psoriasis. Three cases of exfoliative dermatitis with abnormal vitamin C saturation tests showed normal saturation times one month later, when the skin had healed.

The vitamin C content of epidermis is not insignificant, amounting to about 10 mg./100 g. wet weight of tissue (Priestley and Foster, 1959). Comparison with the ascorbic acid content of vitreous humour (30 mg.%) and that of muscle (2 mg.%) shows that epidermis is moderately rich in this vitamin and since it is reasonable to assume that this substance plays an important part in the physiology of normal skin, it is not surprising that the skin's requirements are increased in exfoliation.

Despite the importance of vitamin C to the economy of the living cell, demonstrable abnormalities of biochemical function in the human are confined to abnormalities of tyrosine metabolism, the significance of which is obscure. The scorbutic adult patient excretes normal quantities of tyrosyl derivatives in the urine, but when he is given 20 g. of tyrosine a day he excretes very large quantities of tyrosyl derivatives—from seven to fifteen times the normal amount (Rogers and Gardner, 1949). Similar findings have been reported for the scorbutic guinea-pig (Sealock and Silberstein, 1940), monkey (Salmon and May, 1953), and child (Huisman and Jonxis, 1957; Morris, Harpur and Goldbloom, 1950).

In exfoliative dermatitis, a different pattern is seen, namely a high "tyrosyl" excretion on an ordinary diet and no abnormal increase when loaded with tyrosine. Despite the fact that the vitamin C saturation time is abnormal in exfoliative dermatitis, the increased excretion of tyrosyl derivatives cannot be due to the same disturbance of tyrosine metabolism as in scurvy. Its origin is not clear and further speculation unsupported by more facts is idle.

We do not imply any aetiological relationship between vitamin C and exfoliative dermatitis, but since it appears from our findings that the requirements of the vitamin are increased in this condition, it would seem reasonable to ensure an adequate supply of it to sufferers from extensive skin disease.

SUMMARY.

The ascorbic acid saturation test was carried out in cases of exfoliative dermatitis, eczema, psoriasis, varicose eczema and varicose ulcer.

The saturation time was increased in all except varicose ulcer and was particularly prolonged in exfoliative dermatitis.

Blood ascorbic acid levels appeared to be low in exfoliative dermatitis but since they started to rise as soon as vitamin C was given, the abnormal saturation times did not seem to result from disturbed absorption of ascorbic acid from the gut.

The urinary excretion of tyrosyl derivatives was abnormally high in exfoliative dermatitis but the response to large doses of tyrosine was normal.

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THE SODIUM AND POTASSIUM CONTENT OF THE EPIDERMIS IN ECZEMA, PSORIASIS AND LICHEN SIMPLEX.

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Few references are available in the literature concerning the sodium and potassium content of normal human epidermis. Estimations of the electrolytes in the epidermis of diseased skin using modern methods do not appear to have been reported.

The present investigation was carried out in order to compare the electrolyte content of normal epidermis with that in three common skin disorders. A series of estimations of the sodium and potassium in normal human epidermis is reported and the changes occurring in eczema, psoriasis and lichen simplex chronicus are described. An attempt is made to assess the significance of the results obtained.

INVESTIGATION.

Normal human skin was obtained from two sources.

(i) Operation carried out under general anaesthesia. Skin was taken from an area as far distant as possible from any pathological process. Most of the specimens were obtained from the breast, leg or abdomen.

(ii) Biopsy specimens from normal subjects taken under wide ring block local anaesthesia with procaine 0.5%. Skin from cases of eczema, psoriasis and lichen simplex chronicus was removed in a similar manner. Cases of eczema were selected which were in an acute or subacute stage and were mostly of the discoid type. Specimens from cases of psoriasis were obtained during the active stage before treatment had been commenced and also from lesions which were healing. All cases of lichen simplex showed the classical appearance of lichenification and had been present for many months.

As much subcutaneous fat as possible was removed from each specimen which was then thoroughly washed in distilled water to remove surface contamination. The epidermis was separated from the dermis by a modification of the heat method of Baumberger (1942) and the weight of the epidermis determined (wet weight). Each specimen was dried in a hot air oven at 120° C. to constant weight (dry weight). Extraction of the electrolytes was carried out by digestion in 1 ml. of concentrated nitric acid (M.A.R.), distilled water added to known volume and the sodium and potassium determined by means of flame photometry (EEL flame photometer). When sufficient tissue was available, duplicate determinations were performed on each specimen.

RESULTS.

The sodium and potassium content of normal epidermis is shown in Table I (local anaesthesia) and Table II (general anaesthesia). Table III gives the electrolyte content of the epidermis in eczema, and Table IV the values obtained in eczematous epidermis from which the stratum corneum had been removed.

The results obtained in psoriasis are given in Table V and those in lichen simplex in Table VI. A statistical analysis of all results was carried out and is summarized in Table VII. This showed that no significance could be attached to the observed difference in the sodium content of the epidermis in any of the conditions under investigation. Comparison of the potassium content of the epidermis of normal skin with that of eczema, psoriasis and lichen simplex showed significant differences; these are listed in Table VIII.

The use of procaine 0.5% as the local anaesthetic did not invalidate the

TABLE I.—*Normal Epidermis (local anaesthetic).*

No.	Sex.	Age (years).	Site.	Wet weight (mg.).	Dry weight (mg.).	mEq./Kg. Wet epidermis.	
						Na.	K.
1 .	F.	55	Leg	16.6	6.0	80.0	26.2
2 .	"	39	"	34.2	12.3	101.0	26.9
3 .	"	45	"	7.8	2.6	97.5	39.3
4 .	"	20	"	5.2	1.8	75.5	32.0
5 .	"	42	"	10.1	3.7	80.4	40.3
6 .	M.	51	"	5.8	2.0	70.6	35.1
7 .	"	54	"	6.4	2.4	68.6	40.3
8 .	"	30	"	7.2	2.6	70.4	49.8
9 .	F.	57	Breast	7.5	2.6	139.0	25.2
10 .	"	40	"	6.2	2.4	94.0	30.6
11 .	M.	61	Chest	5.4	1.8	93.1	42.7
12 .	"	56	Arm	5.1	1.9	75.2	31.2

Na : 68.6 to 139.0 mEq./Kg. (mean 87.1).

K : 25.2 to 49.8 mEq./Kg. (mean 34.9).

TABLE II.—*Normal Epidermis (general anaesthetic).*

No.	Sex.	Age (years).	Site.	Wet weight (mg.).	Dry weight (mg.).	mEq./Kg. Wet epidermis.	
						Na.	K.
1 .	M.	62	Leg	11.4	4.4	114.5	31.5
2 .	"	29	"	11.8	4.6	74.0	68.3
3 .	"	50	"	12.4	4.1	82.1	41.3
4 .	"	36	"	7.2	2.8	72.8	61.1
5 .	"	51	"	10.4	3.4	65.4	52.9
6 .	F.	63	"	7.8	2.6	97.5	40.8
7 .	"	45	"	27.8	10.4	72.7	50.0
8 .	"	51	Breast	78.3	29.6	92.8	30.0
9 .	"	37	"	11.0	4.0	75.3	63.0
10 .	"	39	"	14.5	5.8	109.0	37.0
11 .	"	20	"	15.2	5.4	128.0	37.2
12 .	M.	30	Chest	7.2	2.7	74.3	32.1
13 .	"	42	"	10.4	3.6	74.6	63.0
14 .	"	40	Abd.	22.4	7.8	53.7	54.9
15 .	"	55	"	11.8	4.2	52.7	52.1
16 .	F.	30	"	18.2	7.1	59.0	63.2
17 .	M.	25	Penis	17.3	4.6	61.5	65.0
18 .	"	2	"	19.6	5.4	70.8	65.4
19 .	"	17	Ear	15.7	4.9	56.5	73.4
20 .	F.	9	"	12.2	4.0	103.3	75.0

Na : 52.7 to 128.0 mEq./Kg. (mean 79.5).

K : 30.0 to 75.0 mEq./Kg. (mean 52.8).

TABLE III.—*Eczema*.

No.	Sex.	Age (years).	Type.	Stage.	Site.	Wet weight (mg.).	Dry weight (mg.).	mEq./Kg. Wet epidermis.	
								Na.	K.
1.	M.	52	Discoid	Acute	Leg	5.2	1.4	68.5	60.5
2.	"	47	"	"	"	12.6	3.6	125.4	78.8
3.	"	17	"	"	"	6.2	2.2	56.5	56.8
4.	F.	65	"	"	Arm	5.4	1.7	68.4	35.4
5.	M.	53	"	"	"	7.4	2.4	70.0	60.6
6.	"	50	"	"	Chest	7.0	2.0	62.0	84.3
7.	"	62	"	Sub-acute	Leg	5.4	1.8	144.9	90.2
8.	"	36	"	"	"	10.2	2.8	75.6	112.0
9.	"	47	"	"	"	10.2	3.1	70.1	94.0
10.	"	45	"	"	"	9.2	3.0	54.3	101.7
11.	"	50	"	"	"	9.0	2.7	84.2	70.3
12.	F.	61	"	"	"	6.4	2.0	92.4	81.5
13.	M.	35	"	"	Arm	4.0	1.2	93.2	110.0
14.	"	60	Stasis	"	"	7.4	2.8	58.7	100.5
15.	F.	47	"	"	"	3.6	1.0	63.0	72.1

Na : 54.3 to 144.9 mEq./Kg. (mean 79.1).

K : 35.4 to 112.0 mEq./Kg. (mean 80.6).

TABLE IV.—*Eczema (stratum corneum removed)*.

No.	Sex.	Age (years).	Type.	Stage.	Site.	Wet weight (mg.).	Dry weight (mg.).	mEq./Kg. Wet epidermis.	
								Na.	K.
1.	M.	59	Discoid	Acute	Leg	10.3	3.1	63.0	89.6
2.	"	30	"	Sub-acute	"	8.2	2.7	60.4	93.2
3.	"	25	"	"	"	11.5	3.8	68.0	89.2
4.	"	35	"	"	"	10.5	3.2	74.5	87.9
5.	"	52	"	"	Chest	3.5	1.1	93.1	87.9
6.	F.	40	Sebor- rheic	"	Leg	3.4	1.1	94.6	73.4

Na : 60.4 to 94.6 mEq./Kg. (mean 75.6).

K : 73.4 to 93.2 mEq./Kg. (mean 86.8).

TABLE V.—*Psoriasis*.

No.	Sex.	Age (years).	Stage.	Site.	Wet weight (mg.).	Dry weight (mg.).	mEq./Kg. Wet epidermis.	
							Na.	K.
1.	M.	29	Acute	Trunk	4.6	2.0	89.8	128.2
2.	"	24	"	"	9.0	3.1	62.1	94.2
3.	"	46	"	"	21.6	6.3	65.2	85.0
4.	"	27	"	"	11.2	3.2	90.4	106.3
5.	"	29	"	Leg	10.8	3.5	64.4	76.0
6.	F.	63	"	"	13.0	4.2	65.7	100.5
7.	M.	24	"	Arm	11.4	3.2	71.3	127.0
8.	"	56	Sub-acute	Trunk	8.5	3.6	88.0	54.3
9.	"	29	"	"	7.5	2.7	75.4	63.2
10.	"	49	"	"	9.4	3.0	66.9	81.8
11.	F.	39	"	"	6.0	2.6	80.0	89.9
12.	"	33	"	"	8.1	2.6	56.1	63.3
13.	M.	57	"	Leg	9.0	2.8	57.9	85.4
14.	"	64	"	"	6.4	3.0	67.9	43.8
15.	"	57	"	"	4.7	2.2	91.2	88.6
16.	"	57	"	"	8.0	3.0	97.3	86.5
17.	"	59	"	"	15.0	4.9	64.8	71.4
18.	F.	42	"	"	15.8	4.7	69.4	76.3

Na : 56.1 to 97.3 mEq./Kg. (mean 73.5).

K : 43.8 to 128.2 mEq./Kg. (mean 84.5).

TABLE VI.—*Lichen Simplex Chronicus.*

No.	Sex.	Age (years).	Site.	Wet weight (mg.).	Dry weight (mg.).	mEq./Kg. Wet epidermis.	
						Na.	K.
1	M.	40	Leg	2.2	0.8	93.1	73.2
2	"	61	"	13.4	4.6	101.0	78.3
3	"	53	"	9.8	3.2	60.8	75.8
4	"	25	"	7.5	3.0	64.2	96.1
5	"	42	"	9.1	3.4	79.0	83.2
6	"	51	Arm	9.4	3.2	89.2	84.6

Na : 60.8 to 101.0 mEq./Kg. (mean 81.2).

K : 73.2 to 96.1 mEq./Kg. (mean 81.8).

TABLE VII.—*Epidermal Sodium and Potassium in mEq./Kg.
(Wet weight in various groups.)*

Group.	Description.	No. of cases.	Sodium.			Potassium.		
			M.	S.D.	C.V.	M.	S.D.	C.V.
1	Normal (local anaesthetic)	12	87.1	19.8	22.7	34.9	6.3	18.1
2	Normal (general anaesthetic)	20	79.5	19.3	24.4	52.8	13.4	25.4
3	Eczema	15	79.1	25.6	32.4	80.6	21.8	27.0
4	(Acute)	7	73.3	23.3	31.7	64.1	16.3	25.5
5	(Sub-acute)	8	84.1	27.9	33.2	95.1	14.2	15.0
6	(less stratum corneum)	6	75.6	15.1	20.0	86.8	6.6	7.6
7	Lichen simplex	6	81.2	15.5	19.1	81.8	8.2	10.3
8	Psoriasis	18	73.5	13.4	18.2	84.5	21.2	25.1
9	(Acute)	7	72.6	10.8	14.8	102.0	18.5	19.0
10	(Sub-acute)	11	74.1	13.5	18.2	73.1	15.5	21.0

S.D. : standard deviation.

C.V. : percentage coefficient of variation.

TABLE VIII.—*Significance of Observed Differences.*

Groups compared.		Sodium.		Potassium.	
		P (Student's <i>t</i> test).	Significance.	P (Student's <i>t</i> test).	Significance.
1	vs. 2	<0.4	Not significant	<0.001	Significant
3	vs. 4	<0.5	" "	<0.01	"
1	vs. 6	<0.6	" "	<0.001	"
1	vs. 7	<0.3	" "	<0.001	"
8	vs. 9	<0.9	" "	<0.01	"
1	vs. 10	<0.3	" "	<0.001	"

results for sodium since they are in general agreement with those obtained with specimens taken under general anaesthesia. Also, since procaine does not contain any potassium no error was introduced.

DISCUSSION.

Estimations of sodium and potassium in normal human epidermis have been carried out by several workers. Zheutlin and Fox (1950) reported the following results.

Sodium : 32 to 62 (mean 46.1) mEq./Kg. of wet epidermis.

Potassium : 69 to 96 (mean 80.5) mEq./Kg. of wet epidermis.

These were in general agreement with those reported earlier by Suntzeff and Carruthers (1945) viz.

Sodium : 0.102 to 0.162 (mean 0.122) mg./100 g. of epidermis.
= 44 to 62 (mean 54) mEq./Kg. of epidermis.

Potassium : 0.275 to 0.377 (mean 0.322) mg./100 g. of epidermis
= 70 to 96 (mean 82) mEq./Kg. of epidermis.

The values for sodium in normal epidermis found in the present study (79.5 and 87.1 mEq./Kg.) are higher than those referred to above. Contamination of the surface of the skin and apparatus with sodium is difficult to avoid unless elaborate precautions are taken. This no doubt explains some of the highest values which were obtained for sodium. The trauma due to biopsy might also affect the distribution of the electrolytes, sodium tending to enter the cells to replace potassium.

The potassium content of normal epidermis (50.8 and 34.9 mEq./Kg.) was found to be lower than that reported by other workers. Preliminary experiments showed that the potassium content fell if the epidermis was not separated from the dermis within a relatively short period of time (up to about 4 hours). If the separation was delayed more than 12 hours, low values for potassium were obtained, probably due to the ion passing into the dermis. This might account for some of the lower values for potassium but by no means all, since separation of the epidermis was carried out within 4 hours of obtaining the specimen whenever possible. As in the case of sodium, trauma associated with biopsy may have disturbed the electrolytic equilibrium and potassium may have escaped from the cells during the operative procedure. The traumatic effect would be expected to be greater in small specimens taken under local anaesthesia and this might explain the lower potassium values found compared with those in specimens taken under general anaesthesia.

Earlier work on the potassium content of the epidermis carried out in this laboratory using the same method as that described by the present author, also showed lower values for potassium than those reported by other workers.

Most of this work was performed on specimens taken under general anaesthesia and hence the traumatic effects, referred to above, were probably minimal (Tickner, personal communication).

Comparison of the sodium content of normal and eczematous epidermis failed to reveal any significant differences. The potassium content of the epidermis in eczema is raised and this is statistically significant. Since potassium is the main intracellular ion, it is to be expected that an increase in epidermal cells would result in a higher potassium content. That acanthosis is the most likely cause for the higher potassium content in psoriasis has been suggested by Dorffel (1930).

A series of eighteen determinations of sodium and potassium was carried out in psoriasis in an attempt to confirm this observation. The results given in Table V indicate that the potassium content of psoriatic epidermis is significantly raised. More detailed analysis of these results shows that the potassium content is highest when the psoriasis is in an active, untreated stage and falls when the lesions are involuting. Similar experiments performed with six specimens of skin from patients with lichen simplex chronicus showed a raised potassium content compatible with the acanthosis found in this condition (Table VI).

In view of these findings, it was thought possible that the raised potassium content of eczematous epidermis could also be due to acanthosis. The latter varies with the stage of the eczematous process, being slight in the acute, and moderate in the subacute phases of the disease. Further analysis of the results obtained in eczema (Table III) shows that lower potassium values are found when the eczema is in an acute vesicular stage, and rise when the process becomes subacute. A further experiment was carried out in an attempt to show that it is the hypertrophy of the prickle cell layer which is responsible for the raised potassium content of the epidermis in eczema. The stratum corneum was stripped off lesions in six cases of discoid eczema by using Sello-tape following the method of Pinkus (1951). Preliminary experiments had shown that it was possible to remove all the stratum corneum and this was confirmed histologically; only a few parakeratotic cells remained. Biopsies were performed on the stripped eczematous lesions under local anaesthesia and the electrolytes determined in the specimens obtained as before. The results in Table IV show that the potassium content was raised in all six cases. As the specimens consisted almost entirely of hypertrophied prickle cells, it seems likely that acanthosis is responsible for the high potassium content.

SUMMARY.

Estimations of the sodium and potassium content of human epidermis in normal individuals and of patients with eczema, psoriasis and lichen simplex chronicus are reported.

The sodium content of the epidermis in the diseases investigated did not differ significantly from that in normal skin.

The potassium content of the epidermis was found to be raised in these diseases, probably due to acanthosis.

I should like to thank Dr. A. Tickner for suggesting this investigation and giving his helpful advice. I am indebted to the physicians of St. John's Hospital for kindly allowing me to perform biopsies on patients under their care.

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OBITUARY.

ALLISON D. McLACHLAN, M.D.

Dr. Allison David McLachlan died on 30 June 1960, at the age of seventy-six. He had actively practised dermatology in Glasgow and in the West of Scotland for more than thirty years.

His appointments had included visiting consultancies in diseases of the skin at Stobhill Hospital, the Victoria and Western Infirmarys of Glasgow and the Royal Infirmary, Falkirk, and he had been in charge of wards in Stobhill and in the Western Infirmary until his retirement in 1950. From 1936 to 1950 he had been a Lecturer in Dermatology at the University of Glasgow.



McLachlan, Dr. J. Ferguson Smith and the late Dr. W. Herbert Brown formed the triumvirate of dermatologists who dominated the Glasgow scene in the Twenties and Thirties, and he lived to see many of their schemes for the improvement of dermatological services in Scotland brought at last to fruition after the conclusion of the 1939-45 war. He was not a prolific contributor of articles to medical periodicals, his best known contributions being in all probability those published in this journal on fractional gastric analysis in diseases of the skin (with W. Herbert Brown and M. S. Smith) and on Kaposi's varicelliform eruption. Despite this, he was the source of inspiration of many theses for the degree of Doctor of Medicine subsequently produced by his assistants, all of whom owe him a great debt of gratitude for the encouragement and warm friendship shown to them over the years during which they were so fortunate as to be associated with him.

A man of kindly nature, of a pawky humour and of considerable stature as a physician and specialist, Allison McLachlan will be sadly missed by his many colleagues and friends. Our sympathy is extended to his wife, daughter and son in their bereavement.

A. G. F.

BRITISH ASSOCIATION OF DERMATOLOGY.

THE FORTIETH ANNUAL MEETING.

THE Fortieth Annual Meeting of the Association was held in London from 6 to 9 July under the presidency of Dr. L. Forman. It incorporated a Franco-British Reunion in which a score of French colleagues participated.

Sir Archibald Gray retired as Treasurer after serving for twenty years. He received a standing ovation and was presented with a cheque as a token of the Association's appreciation. Dr. F. Ray Bettley, who retired as Editor in 1958, was given a silver salver in recognition of his ten years' service.

The following were elected to serve on the Executive Committee for 1960-61 :

President and Hon. Assistant Editor :	Dr. G. W. Bamber.
Immediate Past President :	Dr. L. Forman.
Vice-President :	Professor G. H. Percival.
Hon. Treasurer :	Professor J. T. Ingram.
Hon. Secretary :	Dr. D. I. Williams.
Hon. Editor :	Dr. P. J. Hare.

Ordinary Members of the Committee :

Dr. J. M. Beare.	Dr. H. J. Wallace.
Dr. F. Ray Bettley.	Dr. R. P. Warin.
Dr. G. A. Hodgson.	Dr. G. C. Wells.
Dr. R. M. B. MacKenna.	Dr. D. S. Wilkinson.
Dr. G. B. Mitchell Hegg.	

Dr. Georges Garnier was elected an Hon. Foreign Member, and the following as Ordinary Members :

Dr. G. A. Beck.	Dr. G. Holti.
Dr. I. McCallum.	Dr. K. D. Crow.
Dr. J. K. Morgan.	Dr. J. G. Holmes.
Dr. J. D. Everall.	Dr. J. Curry.

The scientific business of the meeting was opened by Dr. Arthur Rook with a stimulating paper on the theoretical and clinical aspects of autoimmune disease. Mr. W. E. Parish then described the strict experimental criteria adopted in its detection. In two of many cases of eczema investigated by him, positive evidence of autosensitization was obtained. Professor P. G. H. Gell described experiments showing that conjugates of a sensitizing agent with homologous protein were capable of inducing contact sensitization more readily than the simple agent, or the latter coupled with foreign protein. Dr. G. Holti produced evidence that a single injury to the skin, such as that from U.V.L., caused long standing impairment of the performance of the cutaneous blood vessels. Dr. George Clarke showed colour motion pictures of onchocerciasis, a worthy effort under any circumstances and surely especially praiseworthy as they were made by Dr. Clarke himself during his busy clinical life in Nigeria. Dr. Fairburn spoke on regeneration patterns in moles and Dr. I. W. Whimster made out a case for the neural control of the growth of cellular

naevi. Dr. A. Jarrett explained the histological rationale of the treatment of psoriasis with vitamin A and triamcinolone.

A group of papers by Dr. A. Porter, Dr. I. A. Magnus and Dr. A. Tickner covered the theoretical, experimental and clinical aspects of the investigation of cases of light sensitization. Dr. P. F. D. Naylor described preliminary work on the measurement of the oxygen tension in skin by an electrical method, and Dr. J. C. Lawrence spoke on experiments with a microrespirometer on the effect of various agents on the metabolism of small implants of skin.

Due to pressure of time, Dr. C. F. H. Vickers' paper on the biochemical differential diagnosis of collagen diseases was postponed until the Friday morning preceding the Franco-British Meeting. The latter comprised alternate papers from French and British contributors, beginning with one from Professor Degos and Dr. J. Civatte on unusual histological appearances in Duhring's disease; they described the occurrence of intra-epidermal and sometimes subcorneal vesicles which they felt did not invalidate the conception of this disease as an entity. Dr. H. Haber described the histology of eleven cases of alopecia mucinosa (Pinkus). *In absentia*, a paper was read on behalf of Drs. Duperrat and Bret, on a papulo-vesicular eruption common in neonates. Dr. R. W. Riddell spoke on his work with Dr. J. G. Pepys on antibodies to fungi. Professor M. H. Thiers described a refinement of the technique of Barber and Oriel for the isolation of urinary products significant in the treatment of allergic disorders. Dr. G. C. Wells' paper on phosphorylase in human skin included aptly culinary details. Professor P. Temine discussed the significance of visceral manifestations of Kaposi's disease. The day was rounded off by a spirited performance by Dr. Aitken Ross who described the history and clinical manifestations of "faun tail" in French of admirable sonority. The clinical case meeting was held in the medical out-patients' department of St. Thomas' Hospital. Throughout the Franco-British Meeting Dr. J. Civatte and Dr. F. Cottenot served as interpreters with amazing facility.

The social programme included the annual Dowling-McCaw golf tournament, won by Dr. Thomas Cochrane. The Bowers-Sneddon sailing cup remained in the clearly competent hands of Dr. Bowers. The President held a reception at the Royal Society of Medicine, and the annual dinner took place at the Grocers' Hall.

The next Annual Meeting will be held in Liverpool on 6, 7 and 8 July, 1961.

NORTH OF ENGLAND DERMATOLOGICAL SOCIETY.

Meeting 8 March 1960 at Leeds.

Favus.—Dr. ALLAN BIGHAM.

A family consisting of a mother and six children (4 girls and 2 boys) all suffer from favus.

The mother had previously been diagnosed as severe seborrhoeic dermatitis and had suffered from it since childhood.

All showed fluorescence under Wood's light and the diagnosis was confirmed microscopically and by culture by Dr. C. LaTouche. The ages of the children range from one year eight months to eleven years. The other two members of the family, the father and a daughter aged 13, in a children's home, have not yet been examined.

Only one of the girls showed typical scutulae.

The following members of the family have so far been examined and confirmed to have favus.

Duration according to Mother's statement:

(i) Mother <i>aet.</i> 36	Since childhood.
(ii) Alice <i>aet.</i> 11	2 years.
(iii) Pamela <i>aet.</i> 9	6 months.
(iv) Stephen <i>aet.</i> 8	2 weeks.
(v) Sandra <i>aet.</i> 7	6 months.
(vi) Leslie <i>aet.</i> 5	2 weeks.
(vii) Doreen <i>aet.</i> 1-8/12. . . .	2 weeks.

No faith is placed in the accuracy of duration amongst the children.

Treatment has been instituted with griseofulvin.

Candidiasis.—Dr. A. J. E. BARLOW.

A boy aged 3 years 9 months.

History.—At birth he weighed 6½ lb. and is said to have been a normal child. Developed oral thrush at three months, followed by recurrent attacks of bronchitis. He was first admitted to the children's unit at the age of nine months for an acute respiratory infection, which responded to antibiotics, his chest skiagram (March 1957) being clear. He developed, however, an intermittent low pyrexia for several months and a scurfy "eczema" of the scalp and groins. Since that time his weight has increased only slightly. His general development has been retarded and he has been prone to respiratory infections characterized by pyrexia, extreme dyspnoea and cyanosis with diffuse symmetrical stippling of the lung pattern on X-ray examination. He has had continual oral thrush and well demarcated areas of scurfy erythema on the scalp, trunk and hands, in all of which sites *Candida albicans* has been demonstrated.

Investigations.—Routine blood chemistry, including serum calcium is within normal limits; faecal fat and duodenal juice are normal. He has, from time to time, shown varying degrees of secondary anaemia. His haemoglobin is now 87%.

Present condition.—Debilitated under-developed child aged three years. Weight 22 lb.

Confluent scurfy erythema of the whole scalp, sides of face and neck, with similar well-defined areas on the arms, trunk and legs. Both palms are thickened and all finger nails are opaque, the nail plates being up to 1 cm. thick. The tongue and buccal mucosa, and to some extent the lips, are covered with a confluent thrush.

Treatment.—No improvement on nystatin orally and by inhalation, amphotericin B, or griseofulvin.

Purpuric Parapsoriasis.—Dr. F. F. HELLIER.

A boy aged 12.

First seen November 1957 with a history of a rash for six months on the buttocks, backs of thighs and a little on the feet. At the time there were finger-tip size red macules over most of the trunk and on the legs, especially well marked over the buttocks. Many lesions showed definite faint purpuric dots. General examination was negative as was blood count, serum proteins.

He was kept under observation and the condition gradually spread. By November 1959 the lesions had become more scaly with rather larger patches from 1-3 cm. diameter. Some of them looked rather psoriasiform and some had a definite poikilodermatous appearance.

A large series of investigations, which included Wassermann reaction, prothrombin time, serum cryoglobulins, liver tests, serum electrophoresis, was normal.

A biopsy specimen in 1958 showed some non-specific perivascular infiltrate in the dermis with a little haemorrhage here and there. There was some invasion of the epidermis by lymphocytes. He has not responded to any form of treatment.

CORRESPONDENCE.

*The Editor, 'The British Journal of Dermatology',
85 Harley Street, London, M.1, England.*

SIR,—I read Mr. Tickner's and Dr. Basit's findings in your April 1960 Journal with great interest.

However, from a personal communication from Mr. Tickner, it appears that our series are not strictly comparable. All their cases had their liver function tests well within four weeks of exfoliation and usually much earlier. In my series, all cases which had exfoliated up to three weeks were excluded. Except for the first case, all my patients had their liver function tests at, or soon after admission; however, all of these cases had been exfoliating three weeks or longer, before they were admitted to my ward. One case exfoliated three weeks and two days, eight cases between four to six weeks, and one case seven months before their liver function tests were undertaken.

I stressed in Para. 5 of the discussion, and in Para. 7 of my summary of my paper that cases which had suffered from exfoliative dermatitis of more than three weeks' standing, may develop a degree of liver damage which, however, would be of only moderate extent. In 1954 a further series of twenty cases were observed which had supported the previous findings.

Unfortunately with the advent of time, the cheapness and easy availability of cortico-steroids made it impossible on ethical grounds, to collect a further group of patients which had exfoliated for a sufficiently long time to show constantly abnormal findings.

I remain,
Yours faithfully,
F. BAUER.

Melbourne.

ERRATUM.

Brit. J. Derm., 72/7 (July), p. 260. First line should read "in daylight, and even violet and blue visible light, may influence . . .".

BOOK REVIEW.

SCIENTIFIC DERMATOLOGY*

THE chorus of general approval which seems to have greeted the appearance of this book has not exaggerated its merits. It is a product of the modern tendency to approach medical problems through science, a trend which in dermatology received a considerable impetus by the publication in 1953 of a large work on the physiology and biochemistry of the skin by Stephen Rothman and his co-authors. The new perspective can no doubt be attributed to various influences, such as the rapid growth of the fundamental sciences during the past few decades, perhaps also to the growing

* *Progress in the Biological Sciences in relation to Dermatology*. ARTHUR ROOK, Editor (1960). London: Cambridge University Press. Pp. 480. Price £4 4s.

influence of the Medical and Surgical Units on medical education and almost certainly to a widespread feeling among dermatologists that by and large the approach through the speculative hypotheses has accomplished very little. The new type of literature, whether in monographs, current medical journals or in symposia such as the one under review, is avidly read by dermatologists in all countries and without doubt the new outlook must be reflected before long in standard textbooks on dermatology, as indeed it is in one large relatively new work which appears at present to be by far the most popular of its class.

A course at Cambridge held in September 1958 in the Postgraduate Medical School of the University was the first dermatological enterprise of the new order to be undertaken in Great Britain and it was jointly organised by the Regius Professor of Medicine, Professor J. S. Mitchell and Dr. Arthur Rook. Its Proceedings consist of thirty seven papers on eleven topics and the general discussions which followed each topic. These were selected from a very wide field indeed and include the melanocyte and melanogenesis, cutaneous innervation, histochemical investigations, bacteriology and mycology, psycho-physiological mechanisms, comparative medicine, immunology, inflammation, carcinogenesis and the pharmacology of vasomotor and tranquillising drugs. The great majority of the contributions are summaries of scientific knowledge on the various topics by research workers in Cambridge University and in other British universities, with emphasis on work performed by the speakers themselves.

It could be argued that the majority of those for whose benefit the symposium is primarily intended are in no position critically to appraise the value or validity of the work presented to them and that accordingly the proceedings may serve no very useful purpose. The transcriptions of the tape recordings of the discussions do not give that impression. They are certainly interesting and lively and they appear to be to the point. It would seem even possible that the scientists may at times have gained something of practical use for themselves, at least in learning something about the clinicians' viewpoint.

Interests obviously vary and little would be gained by attempting to discuss and compare individual contributions. One or two articles are too technical for the digestion of one ordinary reader; a few others are easy to assimilate but are weak. The great bulk of the text appears to be of the best class of medical publication, likely to be of absorbing interest to students of dermatology of all ages and presented in a form which is readily accessible to them. This is unquestionably a distinguished and valuable addition to dermatological literature and among British works on the subject it is the first of its kind. Let us hope that it is not to be the last.

G. B. D.

CURRENT LITERATURE.

SOME OBSERVATIONS ON PERIPHERAL BLOOD FLOW DURING ADMINISTRATION OF ETHYLENE DIAMINE TETRACETIC ACID (E.D.T.A.). S. R. MASON, F. S. CALIVA and R. H. LYONS. (1959) *J. invest. Derm.*, **33**, 1.

These authors failed to show any significant changes in finger or toe skin temperatures or blood flow as a result of giving E.D.T.A. intravenously.

One patient with scleroderma showed a significant increase in blood flow. This finding agrees with those previously reported by Rukavina *et al.* (1957). The substance itself is a chelating agent; the improvement shown in cases of scleroderma cannot be explained. A. J.

THE EFFECT OF ACTH AND HYDROCORTISONE ON THE HUMAN SEBACEOUS GLAND. S. J. STRAUSS and A. M. KLYMAN. (1959) *J. invest. Derm.*, **33**, 9.

Biopsies of the cheek were taken before treatment, and after treatment with either ACTH or oral hydrocortisone other biopsies were taken from the identical portion on the contralateral cheek at varying intervals after therapy.

The authors concluded that ACTH produced substantial glandular enlargement in two of three cases; hydrocortisone did so in one of three cases.

(The method employed however does not allow of very accurate assessment of changes in glandular size, and consequently the conclusions cannot be accepted as proven.) A. J.

MEASUREMENT OF THE EFFECT OF TOPICAL ANTIPRURITIC AGENTS ON EXPERIMENTALLY INDUCED PRURITUS. S. G. MACRIS, I. H. BLANK and H. K. BEECHER. (1959) *J. invest. Derm.*, **33**, 15.

A standard degree of pruritus was induced by the use of cowhage applied to a constant area. The effects of various antipruritics were then evaluated. The authors concluded that none of the agents used (these included menthol, hydrocortisone and xylocaine ointments) or their placebos significantly altered the severity or duration of the experimentally induced pruritus. A. J.

CONTACT DERMATITIS DUE TO PHENOTHIAZINE DRUGS. D. GOODMAN and M. M. CAHN. (1959) *J. invest. Derm.*, **33**, 27.

A subject who was sensitive to phenothiazine was also sensitive to allied compounds. It was found that positive skin reactions were obtained only with compounds having a chlorine atom in the corresponding position to the chlorine atom in the phenothiazine molecule. Compounds with other radicles or atoms substituted in the same position gave negative results. A. J.

THE EFFECT OF AGE ON MITOSIS IN HUMAN EPIDERMIS. J. M. THURINGER and A. A. KATZBERG. (1959) *J. invest. Derm.*, **33**, 35.

These workers made mitotic counts on fifty-four samples of abnormal skin from individuals whose age varied from 2 days to 77 years. The mitotic activity of the basal layer showed a sustained increase from birth to old age whilst the spinous layer showed a decrease. The upper region of the epidermis became totally inactive with advancing years. A. J.

THE FATTY ACID COMPOSITION OF THE SKIN SURFACE FAT OF NORMAL HUMAN SUBJECTS. B. BOUGHTON and V. R. WHEATLEY. (1959) *J. invest. Derm.*, **33**, 49.

These workers used gas phase partition chromatography to determine the fatty acid composition of normal sebum. They showed skin surface lipids varied very little over a period of time and that there was no change during menstruation. Between individuals however there was a definite personal variation. They also considered that unsaturated acids were mainly derived from the sebaceous glands, whereas the saturated acids were from the epidermal cells.

(This is an important contribution to our knowledge of the composition of human sebum.)

A. J.